ABSTRACT OF THE DISCLOSURE

The invention provides a method for rapidly, economically and efficiently sequencing and assaying nucleotides in a fluid medium using laser induced fluorescence of antisense probes. The probes can have anionic backbones of reduced negative charge. Suitable probes can include methylphosphonate backbones. When the hybridization complexes and unhybridized probes are separated prior to detection, the fluorescent intensity of the fluid test medium is inversely proportional to the number of mismatches between the probe and target. When the hybridization complexes and unhybridized probes are not separated prior to detection, the fluorescent intensity of the fluid test medium is inversely proportional to the hybridization efficiency of the probes with respect to the target sequence and proportional to the number of mismatches between the probe and target. The method can be used to identify accessible regions in folded nucleotide sequences, to determine the number of mismatched pairs in a hybridization complex, and to map genomes.